## **Supplementary Material**

A combination of bovine serum albumin with insulin—transferrin—sodium selenite and/or epidermal growth factor as alternatives to fetal bovine serum in culture medium improves bovine embryo quality and trophoblast invasion by induction of matrix metalloproteinases

Ayman Mesalam<sup>A,B</sup>, Kyeong-Lim Lee<sup>A</sup>, Imran Khan<sup>A,C</sup>, M. M. R. Chowdhury<sup>A</sup>, Shimin Zhang<sup>A</sup>, Seok-Hwan Song<sup>A</sup>, Myeong-Don Joo<sup>A</sup>, Jae-Hoon Lee<sup>D</sup>, Jong-In Jin<sup>A,E</sup> and Il-Keun Kong<sup>A,E,F</sup>

<sup>A</sup>Department of Animal Science, Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, Gyeongnam Province, Republic of Korea.

<sup>B</sup>Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44519, Egypt.

<sup>C</sup>Department of Chemistry, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, 24420, Pakistan.

<sup>D</sup>Department of Veterinary Science, College of Veterinary Science, Gyeongsang National University, Jinju 52828, Gyeongnam Province, Republic of Korea.

<sup>E</sup>Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Gyeongnam Province, Republic of Korea.

FCorresponding author. Email: ikong7900@gmail.com

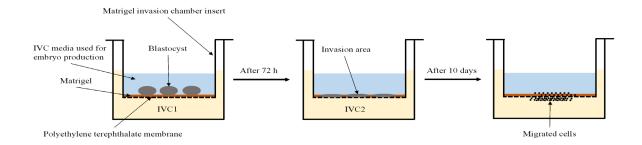


Fig. S1. Schematic diagram showing invasion assay. The Matrigel invasion chamber inserts containing polyethylene terephthalate membrane with 8  $\mu$ m-diameter pores coated with Matrigel (20  $\mu$ g/filter) and placed in each well of a 24-well tissue culture plate. Three blastocysts were located on culture insert and suspended in same media used for embryo production. Under the Matrigel IVC1 medium was added to each culture well and changed to IVC2 after 72 h then refreshed on a daily basis. The invasion assay was performed under a humidified atmosphere of 5% CO2 in air at 37°C for 10 days.